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Development of an Arcellacea (testate lobose amoebae) based transfer function for sedimentary Phosphorus in lakes

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ABSTRACT

Arcellacea (testate lobose amoebae) communities were assessed from 73 sediment-water interface samples collected from 33 lakes in urban and rural settings within the Greater Toronto Area (GTA), Ontario, Canada, as well as from forested control areas in the Lake Simcoe area, Algonquin Park and eastern Ontario. The results were used to: (1) develop a statistically rigorous arcellacean-based training set for sedimentary phosphorus (Olsen P (OP)) loading; and (2) derive a transfer function to reconstruct OP levels during the post-European settlement era (AD1870s onward) using a chronologically well-constrained core from Haynes Lake on the environmentally sensitive Oak Ridges Moraine, within the GTA. Ordination analysis indicated that OP most influenced arcellacean assemblages, explaining 6.5% ($p<0.005$) of total variance. An improved training set where the influence of other important environmental variables (e.g. total organic carbon, total nitrogen, Mg) was reduced, comprised 40 samples from 31 lakes, and was used to construct a transfer function for lacustrine arcellaceans for sedimentary phosphorus (Olsen P) using tolerance downweighted weighted averaging (WA-Tol) with inverse deshrinking ($\mathrm{RMSEP_{jack}}$-77pp; r^2jack = 0.68). The inferred reconstruction indicates that OP levels remained near pre-settlement background levels from settlement in the late AD 1970s through to the early AD 1970s. Since OP runoff from both forests and pasture is minimal, early agricultural land use within the lake catchment was as most likely pasture and/or was used to grow perennial crops such as Timothy-grass for hay. A significant increase in inferred OP concentration beginning ~AD 1972 may have been related to a change in crops (e.g. corn production) in the catchment resulting in more runoff, and the introduction of chemical fertilizers. A dramatic decline in OP after ~AD 1985 probably corresponds to a reduction in chemical fertilizer use related to advances in agronomy, which permitted a more precise control over required fertilizer application. Another significant increase in OP levels after ~AD 1995 may have been related to the construction of a large golf course upslope and immediately to the north of Haynes Lake in AD 1993, where significant fertilizer use is required to maintain the fairways. These results demonstrate that arcellaceans have great potential for reconstructing lake water geochemistry and will complement other proxies (e.g. diatoms) in paleolimnological research.

1. Introduction

Arcellacea (also informally known as thecamoebians (Patterson and Kumar, 2002) or testate lobose amoebae (Mitchell et al., 2008)) are a diverse group of unicellular testate rhizopods that occur in a wide array of aquatic and terrestrial environments from salt marshes to lakes and ponds to peatlands and damp soils (Medioli and Scott 1983, Warner and Chmielewski, 1992; Charman et al., 1998; Beyens and Meisterfeld, 2001; Roe et al., 2001; Riveiros et al., 2007; Van Hengstum, et al., 2008). Among this group, agglutinating forms, primarily Arcellacea, have tests that are highly resistant to decay and thus fossilize well, a characteristic that makes them particularly valuable as paleolimnological indicators (Medioli and Scott, 1983). Arcellaceans are particularly common in Holocene lacustrine and peatland environments from temperate to Arctic regions (Medioli et al., 1990; Patterson and Kumar, 2002). The use of arcellaceans as quantitative paleoenvironmental indicators is most developed in peatland research where they have been used in conjunction with other testate amoebae taxa in the reconstruction of hydrological change (cf. Tolonen, 1986; Mitchell et al., 2001; Swindles et al., 2007, 2009, 2010; Elliott et al., 2011) and as pollution indicators (Gilbert et al., 1999; Payne, et al., 2010; Meyer et al., 2012). Arcellaceans have also been widely reported from lakes (Ellison, 1995; Dalby, et al., 2000; Patterson and Kumar, 2002; Roe and Patterson 2006) where variation in their community assemblages has been used to qualitatively infer parameters such as impact of land use change and forest fires, pH, oxygen, temperature, metal contamination and nutrient fluctuations (Patterson, et al., 1985; McCarthy et al., 1995; Patterson, et al., 1996; Reinhardt et al., 1998; Patterson and Kumar, 2000a, 2000b; Patterson et al., 2002; Reinhardt et al. 2005). In spite of this work, the

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ecology of lacustrine arcellaceans is poorly understood (Asioli et al., 1996) with only a handful of systematic studies carried out that test the response of arcellaceans to specific gradients such as nutrient loading, metal contamination, pH, temperature; road salt contamination (e.g. Escobar et al., 2008; Kihlman and Kauppila, 2009; Roe et al., 2010). Although lacustrine arcellaceans have considerable potential in paleoenvironmental studies, no transfer functions utilizing the group have hitherto been applied to fossil core assemblages.

In a reconnaissance study of environmentally sensitive kettle lakes and ponds across a variety of urban, suburban, agricultural and forested settings from the Great Toronto Area (GTA), Roe et al. (2010) assessed arcellacean-environmental relationships for a large number of variables including water property attributes (e.g. pH, conductivity, dissolved oxygen), substrate characteristics, sediment-based phosphorus (Olsen P (OP)) and 11 environmentally available metals. Roe et al. (2010) recognized a particularly strong association between arcellacean assemblages and OP, indicative of the eutrophic status of many area lakes. They also recognized the influence of locally elevated conductivity measurements on arcellacean communities, which they attributed to road salt inputs associated with winter de-icing operations.

The purpose of this research is to expand upon the work of Roe et al. (2010) by specifically assessing the response of arcellacean communities to a strong OP gradient in selected lakes from the GTA and adjacent control areas of southern and eastern Ontario, as well as undisturbed areas to the north in the Lake Simcoe area and Algonquin Park. The objectives are to: 1) develop a robust arcellacean-based training set for OP loading; and 2) use the modern response data to develop a transfer function to reconstruct past OP levels during the post- European settlement era from Haynes Lake, on the Oak Ridges Moraine.

1.1. Nutrient (phosphorus) loading in GTA lakes

Research carried out on lakes within the rapidly urbanizing parts of the GTA indicates that many water bodies are experiencing a deterioration in water quality and loss of biodiversity as a result of contaminant inputs, particularly external nutrient loading (Howard 1999; Diamond et al. 2002; Howard et al., 2000). Urbanization impacts lake water quality via hydrological changes associated with increased impervious surface cover, which has a major impact on fluxes and storage of nutrients (particularly P) in aquatic ecosystems (Bradford and Maude 2002; Van Metre and Mahler 2005). Phosphorus is a macronutrient required by all forms of life. It is relatively abundant in soils but cycles very slowly through the environment, as it is not present in a naturally occurring gaseous form. Phosphorus is also a major water pollutant as it often acts as a limiting factor in controlling productivity in freshwater environments (Correll, 1999). Phosphorus is often considered the primary cause of eutrophication in lakes, particularly those subjected to point source pollution from sewage and/or agriculture (Kerekes et al., 2004). Algal blooms and eutrophication associated with excess P depletes oxygen levels in aquatic environments, resulting in water quality degradation. Phosphorous was singled out in the Great Lakes Water Quality Agreement (GLWQA) of 1978 under which P detergents were eliminated and the application of phosphate-based agricultural fertilizers was greatly reduced. The agreement also set limits on municipal wastewater treatment plants discharging effluent into the Great Lakes watershed, establishing specific loading targets. In the years following implementation of the GLWQA water quality throughout the Great Lakes watershed improved dramatically. Unfortunately the great strides made in reducing P levels within the boundaries of the GTA following implementation of the GLWQA is rapidly being undone by significant population growth in recent years (Fig. 1). With the population of the GTA expected to reach 7.7 million by 2025 (Ministry of Finance, 2005), total P loading will be an increasingly major problem in many lakes and aquatic communities across the region unless there is a significant investment in infrastructure (Diamond et al., 2002).

Within the GTA P primarily originates from residential sources, including domestic waste, fertilizers and pesticides, and sewage inputs resulting from occasional sewer overflows (TRCA, 2008). Suspended solid inputs associated with urban development are also of concern because these solid particles act as a transport vector for many contaminants, including P (TRCA, 2008). Phosphorus enters lakes across the GTA via multiple pathways, including surface runoff, storm sewer networks and groundwater seepage (Diamond et al. 2002).

2. Materials and methods

2.1. Sampling design

2.1.1. Sampled lakes

In an analysis of arcellacean communities from 21 lakes across an urban, suburban, rural, forest gradient within the GTA, Roe et al. (2010) identified OP as having the greatest influence on assemblage distribution, although other parameters such road salt (conductivity) also influenced assemblage composition in some lakes. To better characterize the influence of OP on arcellacean communities and individual species, 73 samples from 33 lakes deemed to span a continuous P gradient were analyzed. These included 23 lakes from urban to rural environments within the GTA as well as primarily forested areas in Algonquin Park, the Lake Simcoe area and eastern Ontario. This data set was supplemented with samples from 10 lakes from the Roe et al. (2010) GTA study, which contained arcellacean communities primarily controlled by OP (Fig. 1). A summary of the various lake types is found in Supplementary Appendix 1.

2.1.2. Sediment-water interface samples

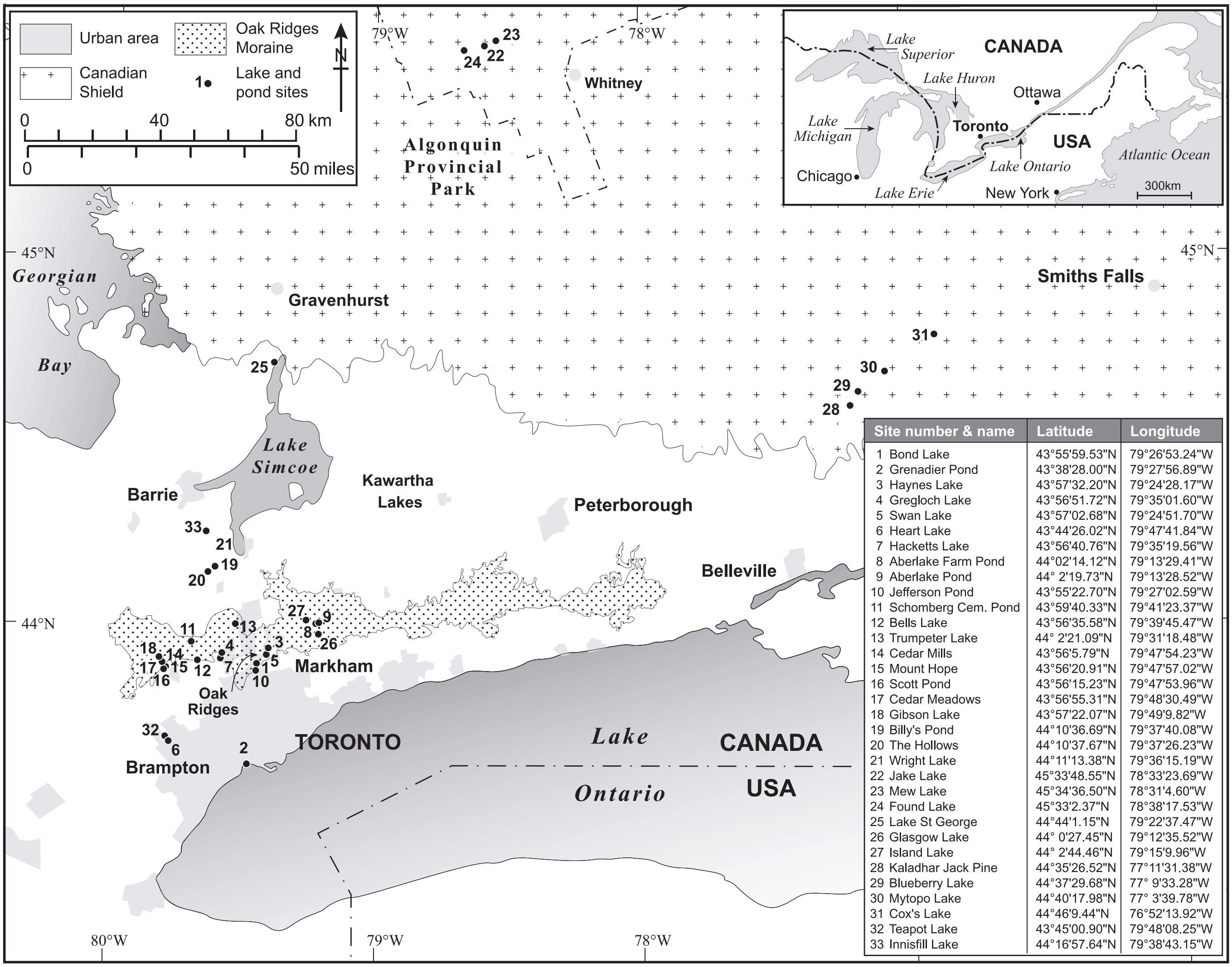
Seventy-three sediment-water interface samples were collected from the study lakes and ponds using an Ekman grab sampler deployed from a boat, retaining the upper 0.5 cm of the sediment for analysis. Since arcellacean assemblages are sensitive to changes in water depth (Patterson and Kumar, 2002) multiple samples were collected from lakes characterized by varying intra-lake limnological conditions. The water depths for each sampling station were determined using a retail market Hummingbird fish finder. Other field-based environmental variables measured included ammonia, chloride, conductivity, dissolved oxygen, nitrate, pH, redox potential and temperature, which were determined using YSI Professional Plus handheld multi-parameter instruments equipped with quatro cables.

2.1.3. Haynes Lake core samples

The Haynes Lake core HYCI (43°57′55″N; 79°24′48″W) was obtained using a Livingstone corer in August, 2005. The 269.5 cm core was subsequently stored at 4 °C in a cold storage facility at Carleton University. The core was logged and also X-rayed using the Kevex instrument at the Canadian Museum of Natural History, Ottawa. The top 32 cm was comprised of very dense, finely layered clay, with a sharp contact to variously laminated and cross-bedded samples below (Fig 2). Only the top 56 cm of core HYCI was included in this research as ^{14}C and ^{210}Pb dating indicates that this interval encompasses the mid-late 19th century European settlement boundary (see Section 2.4 below (Fig. 3)). Thirty-two one-cc samples were subsampled from the upper 56 cm of the core for arcellacean analysis. As described below, the training set for OP derived from the arcellacean communities in the sediment-water interface samples was used to develop a transfer function to estimate OP levels in Haynes Lake from the Pre-European settlement interval through to 2005.

2.2. Laboratory methods

In the laboratory, particle size analysis (% clay, silt and sand) was carried out on each sediment sample using a Beckman-Coulter LS 13 320 Particle Size Analyzer. Sedimentary P was measured to determine



Site number & name	Latitude	Longitude
1 Bond Lake	43°55'59.53"N	79°26'53.24"W
2 Grenadier Pond	43°38'28.00"N	79°27'56.89"W
3 Haynes Lake	43°57'32.20"N	79°24'28.17"W
4 Gregloch Lake	43°56'51.72"N	79°35'01.60"W
5 Swan Lake	43°57'02.68"N	79°24'51.70"W
6 Heart Lake	43°44'26.02"N	79°47'41.84"W
7 Hacketts Lake	43°56'40.76"N	79°35'19.56"W
8 Aberlake Farm Pond	44°02'14.12"N	79°13'29.41"W
9 Aberlake Pond	44° 2'19.73"N	79°13'28.52"W
10 Jefferson Pond	43°55'22.70"N	79°27'02.59"W
11 Schomberg Cem. Pond	43°59'40.33"N	79°41'23.37"W
12 Bells Lake	43°56'35.58"N	79°39'45.47"W
13 Trumpeter Lake	44° 2'21.09"N	79°31'18.48"W
14 Cedar Mills	43°56'5.79"N	79°47'54.23"W
15 Mount Hope	43°56'20.91"N	79°47'57.02"W
16 Scott Pond	43°56'15.23"N	79°47'53.96"W
17 Cedar Meadows	43°56'55.31"N	79°48'30.49"W
18 Gibson Lake	43°57'22.07"N	79°49'9.82"W
19 Billy's Pond	44°10'36.69"N	79°37'40.08"W
20 The Hollows	44°10'37.67"N	79°37'26.23"W
21 Wright Lake	44°11'13.38"N	79°36'15.19"W
22 Jake Lake	45°33'48.55"N	78°33'23.69"W
23 Mew Lake	45°34'36.50"N	78°31'4.60"W
24 Found Lake	45°33'2.37"N	78°38'17.53"W
25 Lake St George	44°44'1.15"N	79°22'37.47"W
26 Glasgow Lake	44° 0'27.45"N	79°12'35.52"W
27 Island Lake	44° 2'44.46"N	79°15'9.96"W
28 Kaladhar Jack Pine	44°35'26.52"N	77°11'31.38"W
29 Blueberry Lake	44°37'29.68"N	77° 9'33.28"W
30 Mytopo Lake	44°40'17.98"N	77° 3'39.78"W
31 Cox's Lake	44°46'9.44"N	76°52'13.92"W
32 Teapot Lake	43°45'00.90"N	79°48'08.25"W
33 Innisfill Lake	44°16'57.64"N	79°38'43.15"W

Fig. 1. Location of sampling sites.

the trophic status of each lake, using the Olsen's phosphorus (Olsen P) extraction method (Olsen et al. 1954). Zhou et al. (2001) demonstrated that this approach provides a measure of bio-available P and is a suitable extraction method for samples of neutral to alkaline pH. Phosphorus concentrations were measured using the phosphomolybdate colormetric technique (Watanabe and Olsen 1965). Total organic carbon (TOC) was measured in each sample using the method described by Hesse (1971). Environmentally available metals known to be important by-products of urban run-off (Ca, Mg, Na, K, Fe, Mn, Zn, Cu, Pb, Cr, Ni) were also analysed from the sediments using an ICP-MS following the US EPA 6010-C methodology (Jones et al., 1987; EPA, 2011) (Supplementary Appendix 2).

Prior to arcellacean analysis 2 cc subsamples were agitated for one hour using a Burrell wrist shaker and subsequently screened with a 250 μm sieve to remove coarse organic debris and then with a 37 μm sieve to remove fine organic and mineral detritus. The 37–250 μm size fraction was selected for arcellacean analysis to allow comparability with other recent lake-based studies (Patterson and Kumar, 2002). The 37–250 μm fraction samples were subdivided into aliquots for quantitative analysis using a wet splitter (Scott and Hermelin 1993). The wet aliquots were subsequently examined under an Olympus SZH10 dissecting binocular microscope (40–80× magnification) until a statistically significant number of specimens were quantified (Patterson and Fishbein, 1989). In most cases >150, and often >250 arcellaceans were counted per sample.

Identification of arcellaceans followed Roe et al. (2010) and was undertaken with reference to standard reference keys (e.g. Medioli and Scott, 1983; Kumar and Dalby, 1998). Lacustrine arcellacean species can display a significant amount of ecophenotypically controlled morphological variability (Medioli and Scott 1983; Medioli et al. 1987; Medioli et al., 1990). The accepted practice by lacustrine researchers is to designate informal infra-subspecic 'strain' names for these ecophenotypes to avoid describing possibly unwarranted new species (Asioli et al. 1996; Patterson and Kumar 2002). Although the International Code of Zoological Nomenclature stipulates that infrasubspecic level designations have no status (International Commission on Zoological Nomenclature, 1999), they are useful for delineating environmentally significant populations within lacustrine environments (Reinhardt et al. 1998; Patterson and Kumar 2000a, 2000b; Roe and Patterson 2006; Escobar et al. 2008; Kihlman and Kauppila, 2009). Arcellacean strain designations were thus quantified during counting (Supplementary Appendices 2, 3). Scanning electron micrograph images of common species and strains were obtained using a Tescan Vega-II XMU VP scanning electron microscope at the Carleton University SEM facility (Fig. 4).

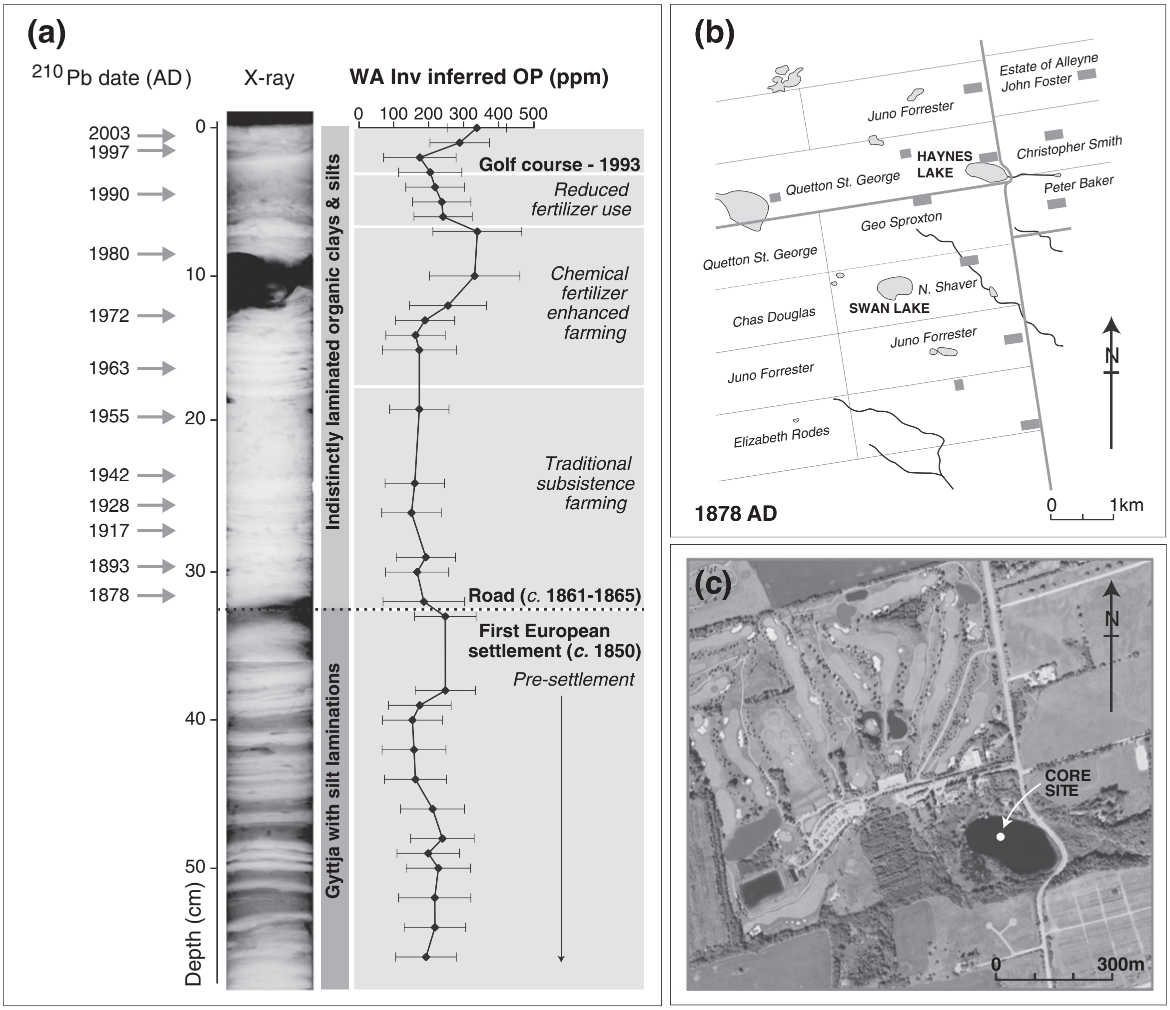


Fig 2. a) X-ray of the top 60 cm of core HL3 with 210Pb derived chronology for top 32 cm and WA Inv inferred OP (ppm) reconstruction. b) Location of Haynes Lake in relation to AD 1878 farms and roads. Buildings indicated by gray rectangles. c) Google Earth aerial imagery of Haynes Lake and surrounding area as of Sept 29, 2009. Diamond Back Gulf and Country Club is upslope to the north. The late 19th century construction of the road around the lake disrupted stream flow into the lake across the flood plain visible to the east. Inset B. Google map of urban development in 2006.

2.3. Statistical methods

Twenty six arcellacean species and strains were identified in the 73 collected sediment/water interface samples and an additional 32 samples from Haynes Lake. The standard error (S_{xi}) associated with each taxon was calculated using the following formula:

$$S_{xi} = 1.96\sqrt{\frac{F_i(1-F_1)}{N_i}}$$

where F_i is the relative fractional abundance of each taxon and N_i is the total of all the species counts in that sample. This methodology requires that if the calculated standard error is greater than the fractional abundance for a particular species in all samples then that species is not included in successive multivariate analyses (Patterson and Fishbein, 1989). As all arcellacean species were found in statistically significant numbers in at least one sample all were included in the multivariate data analyses.

The 73 samples quantified were also assessed to determine which ones were statistically significant. The probable error (pe) for each of the total sample counts was calculated using the following formula:

$$pe = 1.96\left(\frac{s}{\sqrt{X_i}}\right)$$

where s is the standard deviation of the population counts and X_i is the number of counts at the station being investigated. A sample was judged to have a statistically significant population (SSP) if the total counts obtained for each taxon were greater than the pe (Fishbein and Patterson, 1993). All 73 sediment samples were deemed to have SSP counts. The Shannon Diversity Index (SDI) was used to examine the community diversity of the species found in each sample and provides an indication of the relative health of the lakes and ponds (Shannon 1948). The SDI is defined as:

$$SI. = -\sum_{i=1}^{S}\left(\frac{X_i}{N_i}\right) \times \ln\left(\frac{X_i}{N_i}\right) \tag{1}$$

where X_i is the abundance of each taxon in a sample, N_i is the total abundance of the sample, and S is equal to the species richness of the sample. Environments are considered to be healthy if the SDI falls between 2.5 and 3.5, in transition between 1.5 and 2.5, and stressed between 0.1 and 1.5 (Magurran 1988; Patterson and Kumar 2000b). Low SDI values characterise environments where harsh conditions severely limit species numbers.

The relationships between arcellacean assemblages and measured environmental variables follow environmental gradients, which were assessed by means of several statistical techniques using CANOCO version 4.5 and CANODRAW (ter Braak 1987, 2002;

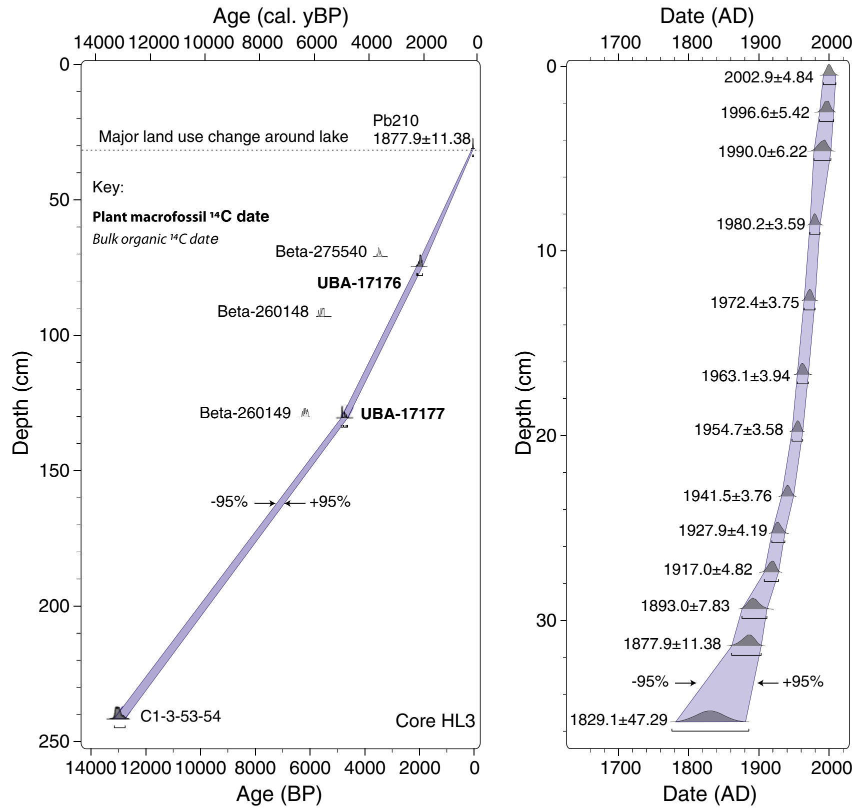


Fig. 3. Age–depth model for Haynes Lake core HL3. Radiocarbon dates were provided by Beta Analytic and the Chrono Centre using the ^{14}C calibration program (Stuiver and Reimer 1993; Stuiver et al., 1998a,b). Age models are based on ^{14}C dates from plant macrophytes (UBA-17176, UBA-17177), a bulk organic sample from near the base of the core (C1-3-53-54) and ^{210}Pb data. Other ages (Beta-260149, Beta-260148 and Beta-275540) based on bulk organic samples are plotted passively. The 95% confidence interval of the regression line is based on the error in ^{14}C ages (solid line bounding gray region). The ^{210}Pb age model for the upper 32 cm of the core where sedimentation rate is higher is presented in right hand figure, the base of which corresponds to the dotted horizontal line in the left hand figure.

Šmilauer, 1992; ter Braak and Šmilauer 2002). The gradient length was determined using Detrended Correspondence Analysis (DCA) of the species data and Detrended Canonical Correspondence Analysis (DCCA) on the species data and selected single environmental variables.

The gradient lengths were consistently long (>2 SD units), suggesting that linear-based methods are not appropriate for these data (Birks, 1995). To overcome this problem the data was transformed using the Hellinger distance, which allows the use of Euclidean-based methods for ordination rather than the chi-squared distance used in Canonical Correspondence Analysis (CCA), which has been shown to sometimes be problematic (Rao, 1995; Legendre and Gallagher, 2001). Redundancy analysis (RDA) was used to investigate taxon–environment relationships in coenospace (Supplementary Appendices 4–6). Forward selection of environmental variables in combination with a Monte Carlo permutation test (999 permutations under a full model) were used to determine the minimum number of variables needed. Variables were removed until a level of significance $p<0.05$ was reached. Summary statistical measures (e.g. concentration, Shannon Diversity Index) were included as supplementary environmental variables. The relative contribution of environmental variables was investigated using a series of partial redundancy analyses (pRDA).

As the multivariate statistical analyses revealed the importance of OP as a major environmental control on arcellaceans in the study sites, transfer functions for OP were developed using weighted averaging (WA), inverse weighted averaging-tolerance (WA-Tol) with classical and inverse deshrinking, weighted averaging with partial least squares (WA-PLS) and maximum likelihood (ML) methods (Table 1). The performance of the models was assessed using r^2, root mean square error of prediction (RMSE), root mean square error of prediction (RMSEP) and maximum bias, calculated as apparent values and with cross-validation based on leave-on-out cross validation (jacknifing). The transfer function models were improved by data screening to remove samples where environmental variables other than OP influenced arcellacean assemblage composition (e.g. low DO near thermocline, elevated conductivity in road side sites contaminated by road salt during winter deicing, coarse substrate influencing productivity, etc.). Samples with a high residual value were removed and a justification of each removal was given (Supplementary Appendix 7). The refined model for P based on WA-Tol (Inv) was applied to a fossil sequence and sample-specific prediction errors were generated through 1000 bootstrap cycles.

2.4. Haynes Lake core chronology

2.4.1. Radiocarbon dating

Radiocarbon dates presented for Haynes Lake core HYC1 are reported in calibrated radiocarbon years before present (cal BP; Table 2; Fig 3). Calibration was carried out using the IntCal09 dendrochronological database for terrestrial material (Reimer et al., 2009). Calibrated bulk organic dates obtained from the top 150 cm of this core were not used in the determination of the sedimentation rate as an ~1500 age offset with calibrated dates obtained from two unidentified and macerated plant macrofossil samples (UBA017176, UBA17177) indicates that “old carbon” may have influenced the bulk dates. Formerly oligotrophic lakes, such as Haynes Lake, where organic concentrations are low, present difficulties for obtaining accurate ^{14}C dates. This is because there are usually only limited identifiable organic remains preserved, making it generally necessary to date bulk sediments (Abbott and Stafford, 1996). Such bulk samples tend to include carbon from various sources that may not be contemporaneous with the sedimentation (e.g. influx of detritus from the lake catchment), resulting in erroneous old ages (Cohen, 2003). The sedimentation rate for the portion of the core deposited below 32 cm was ~0.02 cm/yr.

2.4.2. Lead-210 dating

Thirteen samples from the 0–36.5 cm interval of the core were dated for ^{210}Pb at the St. Croix Watershed Research Station at the University of Minnesota using the alpha spectrometry method, which can accurately determine sediment age through the past 100–150 years (Eakins and Morrison, 1978). Dates and sedimentation rates were calculated using the constant rate of supply model (Appleby and Oldfield, 1978) with confidence intervals determined by first-order error analysis of counting uncertainty (Binford, 1990). The lowermost reliable ^{210}Pb date was an estimate of AD 1877 ± 11.4 at 32 cm in the core (Table 3), which also delineated the base of the fine clay unit that comprised the upper part of the core (Figs. 2, 3). Based on ^{210}Pb results the sedimentation rate through the upper 32 cm of the core was 0.26 cm/yr, a rate more than an order of magnitude higher than the deposition rate lower in the core. Settlers cleared the land around Haynes Lake in 1878 (Canniff 1869; McGill University 2001). The fine clays and accelerated sedimentation in the lake through the upper 32 cm were therefore most likely related to erosional rate changes in the lake catchment brought on by the clearing of trees and the building of the road that skirts the lake to the east (Fig. 2).

3. Results and discussion

3.1. Partial redundancy analysis

Partial redundancy analysis (pRDA) provides a quantification of the proportion of the variance in the arcellacean dataset that can be attributed to the measured environmental variables (Fig. 5; Supplementary Appendix 2) and confirms that several factors influenced the community distribution in the 73 studied samples. Not surprisingly, the most significant control on arcellacean distribution is OP, which explains 6.5% ($p<0.001$) of the total variance. This is a significant result, which highlights the sensitivity of lake arcellaceans to eutrophication. Other significant controls include total organic carbon (TOC at 4.8%; $p<0.003$)), total nitrogen (TN at 3.8%; $p<0.006$) and Mg (4.2%; $p<0.005$).

The concentration of TOC in a lake is a basic parameter for characterizing the amount of organic matter in sediments (Meyers and Teranes, 2001). There is in turn a close correlation between TOC and dissolved organic carbon (DOC) with DOC = 0.9 of the value for TOC (Wetzel, 2001). In humic lakes, which include the majority of lakes in this study, DOC derived from adjacent terrestrial settings dominates most organic carbon (Weyhenmeyer and Karlsson, 2009). This terrestrially derived DOC plays a key role in lacustrine ecosystems because it impacts a number of metrics including lake productivity, community structure and metabolic balances (Jones, 1998; Jansson et al., 2007), as well as the availability of dissolved nutrients and metals (Franco and Heath, 1983). Between lakes the DOC concentration is impacted by factors such as runoff and catchment slope (Sobek et al., 2007). In regions where land use change resulting from urbanization is occurring DOC concentration is directly impacted by point (e.g. wastewater discharge) and non-point (e.g. impervious pavement, lawns) sources (Harbott and Grace, 2005).

As with OP, a primary source of TN to lakes is through terrestrial runoff, which is closely related to urbanization (Guilford and Hecky, 2000). Although both are nutrients the relative concentration and ratio of P and N within a lake can vary significantly and are related to absolute supply of nutrients from external sources, nutrient regeneration rates by micrograzers, mineralization rates by bacteria and loss rates due to sedimentation (e.g. Hecky et al., 1993; Howarth et al., 1996).

The magnesium signal is probably in part derived from Mg-rich groundwater percolating through the coarse textured glacial deposits of the region. Magnesium is also a major constituent of fertilizers and de-icing salts and is common in urbanized areas due to its widespread use in ferrous alloys, electrical and industrial products (Tracy and Baker, 2005).

3.2. Development of a transfer function

The highly significant taxa–environment result for OP indicated in the RDA and pRDA suggests that a transfer function can be developed for OP based on this dataset (Figs. 5 and 6). Detrended Canonical Correspondence Analysis (DCCA) was carried out to establish the gradient length of the data to determine whether unimodal or linear-based regression models would be most appropriate (Birks 1995; Swindles et al. 2007, 2009). As the gradient length was greater than 2σ units unimodal models were selected (Birks, 1995). Several transfer function models were developed based on this training set using weighted averaging (WA), tolerance downweighted weighted averaging (WA-Tol), weighted averaging partial least squares (WA-PLS) regression and Maximum Likelihood (ML) using C2 software (Juggins 2003). The model performance was assessed using the root mean square error of prediction (RMSEP) and the coefficient of determination (r^2) calculated as apparent and leave-one-out cross-validated ('jack-knifed') values (Table 1).

The best performing model with the lowest RMSEP value for OP is WA with inverse deshrinking ($RMSEP_{jack} = 212.70$, $r^2_{jack} = 0.17$) (Table 1). Other, more complex models offer no improvement in model performance (Table 1). Analysis of observed and model estimated variables indicate that there are some outlier samples with high residual values (Fig. 7). As discussed above other variables strongly influenced arcellacean assemblages at some lake sampling stations. To develop a more accurate predictive relationship between arcellacean assemblages and OP, residual outlier samples at the top and bottom 15% of the total range in the predicted residual data were thus removed to improve model performance, leaving 40 in the improved training set (Supplementary Appendix 7). The improved transfer function for OP is based on WA-Tol with inverse deshrinking and has an $RMSEP_{jack}$ of 77 ppm and $r^2_{jack} = 0.68$ (Table 1).

Tolerance and optima values for the 24 arcellacean taxa analyzed are plotted in Fig. 8. All taxa plot out along a distinct gradient with *Difflugia proteiformis* "claviformis" Lamark 1816 being most OP tolerant (up to ~450 ppm) and the soil taxa *Cyclopyxis kahli* (Deflandre 1929) being intolerant of OP levels above ~225 ppm. The general trend of arcellacean taxa tolerance to OP is similar to the findings of Roe et al. (2010), the major difference being that the uppermost thecamobian tolerance in that study was nearly 800 ppm, much higher than found here. Specifically targeting P in the this study, as opposed to utilizing lakes characterized by a broader suite of variables, has resulted in a more refined determination of tolerance ranges for some taxa. For example, the estimated P tolerance range of the very common indicator strain of eutrophism, *Difflugia oblonga* Ehrenberg, 1832 strain "oblonga", has been refined here in the improved training set to ~100–350 ppm as opposed to ~150–600 ppm in Roe et al. (2010). The tolerance ranges for some taxa remain essentially unchanged though (e.g. *Centropyxis constricta* (Ehrenberg, 1843) strain "constricta") with an estimated range of 25–225 ppm in both this study and Roe et al. (2010). Caution must be used when utilizing the estimated ranges for rarer taxa (e.g. *Difflugia bidens* Penard, 1902) as the statistical basis for the OP tolerance levels is lower than for more common taxa.

Prior to application in a transfer function, training sets derived from environmental variables constraining community distribution must be tested against control samples to test their predictive ability (Walker et al. 1991). To test the utility of the transfer function, the WA-Tol with inverse deshrinking model was applied to six samples from four lakes distributed through southern Ontario (Fig. 9). The

WA-Tol Inv transfer function was applied to the contemporary arcellacean data from Teapot Lake, Innisfil Recreation Lake, Bell Lake and Cedar Mills Lake with sample-specific prediction errors being generated by 1000 bootstrap cycles. The results based on these samples indicate that observed OP measurements lie within the bootstrap error range of the model-inferred OP value (Fig. 9). These results from four lakes clearly indicate the applicability of this transfer function for prediction of OP in eutrophic lakes.

3.3. Phosphorus reconstruction for Haynes Lake core

A primary goal of this research has been to elucidate the control that OP loading has had on contemporary lake arcellacean distribution as we assess the relationship between land use, community assemblages and lake sediment and water property data. As the settlement and land use history of the ORM is well known, application of the OP transfer function to a segment of core from Haynes Lake provides an ideal test bed

Table 1
Arcellacean-phosphorus transfer function performance statistics. *WA Inv* weighted averaging with inverse deshrinking, *WA Cla* weighted averaging with classical deshrinking, *WA-Tol Inv* weighted averaging-tolerance downweighted with inverse deshrinking, *WA-tol Cla* weighted averaging-tolerance downweighted with classical deshrinking, *WAPLS* weighted averaging partial least squares (with component number), *ML* Maximum Likelihood. The performance statistics are shown as apparent and jack-knifed (Jack) measures. RMSEP root mean square error of prediction. Values for the best performing model for the whole dataset (WA Inv) and the screened dataset. (WA-Tol (Inv)) are shown in italics.

Total dataset (all samples $n=73$)	WA Inv	WA Cla	WA-Tol (Inv)	WA-Tol (Cla)	WAPLS 1	WAPLS 2	WAPLS 3	WAPLS 4	WAPLS 5	ML
Phosphorus (OP) ppm										
RMSE	*193.38*	353.65	190.48	336.80	193.38	174.18	168.36	163.12	160.34	246.72
r^2	*0.3*	0.3	0.32	0.32	0.3	0.43	0.47	0.5	0.52	0.35
Average bias	*−3.52E−13*	−9.82E−13	4.05E−13	9.84E−13	2.30E−02	6.99E−02	6.85E−02	4.59E−02	2.94E−02	−2.93E+01
Maximum bias	*756.88*	316.17	750.55	362.70	756.93	554.31	571.41	529.63	497.37	935.78
Jack r^2	*0.17*	0.19	0.15	0.18	0.17	0.18	0.14	0.11	0.09	0.28
Jack average bias	*1.76*	5.36	3.06	8.60	1.78	4.80	4.01	1.34	−2.36	−22.11
Jack maximum bias	*844.42*	636.39	837.63	935.19	844.47	681.87	832.28	870.36	945.14	911.89
RMSEP	*212.70*	375.80	215.77	365.68	212.70	220.56	233.69	249.91	264.78	256.43
Dataset after screening ($n=40$)	WA Inv	WA Cla	WA-Tol (Inv)	WA-Tol (Cla)	WAPLS 1	WAPLS 2	WAPLS 3	WAPLS 4	WAPLS 5	ML
Phosphorus (OP) ppm										
RMSE	66.66	76.50	*64.76*	73.67	66.66	57.28	54.68	53.50	52.61	73.09
r^2	0.76	0.76	*0.77*	0.77	0.76	0.82	0.84	0.84	0.85	0.77
Average bias	−6.82E−14	−8.69E−14	*2.15E−13*	2.69E−13	4.01E−02	2.28E−02	8.98E−03	3.94E−03	−5.23E−04	−2.31E+00
Maximum bias	90.71	55.13	*91.12*	58.23	90.81	99.40	91.86	75.92	76.09	79.31
Jack r^2	0.68	0.69	*0.68*	0.69	0.68	0.63	0.61	0.57	0.50	0.61
Jack average bias	1.80	2.06	*1.44*	1.70	1.84	11.63	10.89	14.59	13.45	−2.46
Jack maximum bias	101.53	68.09	*104.08*	73.83	101.61	118.58	114.17	104.73	107.69	97.96
RMSEP	76.65	83.70	*76.63*	81.74	76.65	87.83	88.91	96.84	106.03	93.25

to assess the accuracy of a temporal OP reconstruction (Supplementary Appendix 3).

The laminated and variously cross-bedded interval of the core deposited between 56 and 32 cm (~1200 yBP to AD 1861–1864) was laid down as episodic sedimentary deposits. These beds are probably the result of periodic flooding during large storms, and/or spring freshet, and have as their source the normally small inlet stream immediately to the east of Haynes Lake (Fig. 2c). These sediments are variously clastic and organic rich. The variable nature of the sediments is reflected by the OP reconstruction with intervals characterized by higher organic content having higher reconstructed OP values.

By the beginning of the 19th century European settlers were pouring into southern Ontario, including the ORM, where they established sustenance farms. Roads were built to facilitate settlement starting with the construction of Yonge Street beginning in 1795, which is located 3 km west of Haynes Lake (Stamp, 1991). Roads were laid out in a grid pattern along section boundaries and by ca. 1861–1865 a north–south road was built that skirted the eastern shore of Haynes Lake. Construction of the road resulted in a major change in drainage with the former meandering stream that fed the lake now being restricted to small culverts. This change to the lake inflow resulted in a complete change in sedimentary pattern within the lake with sediments deposited after construction of the road being comprised exclusively finely laminated silts and clays. By AD 1878 the land immediately surrounding Haynes Lake was cleared and settled by several landowners including George Spraxton, Quetton St. George, Peter Baker and Christopher Smith (Canniff, 1869; McGill University, 2001). Land clearance often results in a significant increase in sedimentation rates to impacted water bodies (e.g. Colman and Bratton, 2003), which based on the derived age model for Haynes Lakes, occurred at this site as well (Fig 3). Another common side effect of land clearance and agricultural activity adjacent to lakes is eutrophication (Reinhardt et al., 2005). Although both N and P contribute to eutrophication, P is usually the limiting nutrient in lacustrine trophic systems (Janus and Vollenweider, 1981; Ongley, 1996). Manure produced by cattle, pigs and poultry are characterized by high concentrations of soluble P and are therefore important sources of organic fertilizer throughout the world (Dou et al. 1999). Livestock manure would similarly have served as an important fertilizer for the newly established subsistence farms adjacent to Haynes Lake. Although a large portion of P in manure is soluble in H_2O through precipitation and run-off, the horse drawn manure spreaders that would have been used by settlers leave semi-solid composted clumps of manure on fields, which generally leach P slowly to the environment (Walter et al., 2001). As P is strongly absorbed by soil solids, P runoff from permanently vegetated areas such as pastureland or forests is minimal and largely occurs as traces of orthophosphate (POP_4^{-3}) ions in solution (Evanylo and Beegle, 2006). On the subsistence level farms that were established in the area of Haynes Lake it would have taken a considerable time for P to build up in the relatively nutrient poor soils of recently deforested land to a level high enough to contribute significantly to the eutrophication of Haynes Lake. In contrast, cleared areas where annual crops are grown using conventional tillage, P is moved with eroding soil and immediately

Fig. 4. 1–30. Scanning electron micrographs of selected arcellacean tests from the study lakes. Scale bars = 100 μm. (1) *Arcella vulgaris* Ehrenberg 1830, ventral view, from Bond Lake. (2) *Centropyxis aculeata* (Ehrenberg 1832) strain "discoides", ventral view, from Mytopo Lake. (3) *Centropyxis aculeata* (Ehrenberg 1832) strain "aculeata", specimen with only single spine, from Mytopo Lake. (4) *Centropyxis aculeata* (Ehrenberg 1832) strain "aculeata", ventral view, test constructed of diatoms, from Mew Lake. (4) *Centropyxis aculeata* (Ehrenberg 1832) strain "aculeata", ventral view, test constructed of diatoms, from Mew Lake. (5) *Centropyxis aculeata* (Ehrenberg 1832) strain "aculeata", dorsal view, illustrating that spines are hollow, from Mytopo Lake. (6) *Centropyxis constricta* (Ehrenberg 1843) strain "spinosa", ventral view, from Island Lake. (7,8) *Centropyxis constricta* (Ehrenberg 1843) strain "constricta", oblique and side views, from Swan Lake. (9) *Difflugia urceolata* Carter, 1864 strain "urceolata", side view, from Mew Lake. (10) *Difflugia bidens* Penard 1902, side view, from Mew Lake. (11,12) *Difflugia corona* Wallich, 1864, side and apertural views, from Mew Lake. (13) *Lesqueresia spiralis* (Ehrenberg, 1840), side view, from Mew Lake. (14) *Lagenodifflugia vas* Leidy, 1874, side view showing typical constriction at base of neck, from Mew Lake. (15) *Pontigulasia compressa* (Carter 1864), face view of compressed test showing typical v-shaped depression at base of neck, from Mew Lake. (16) *Difflugia oblonga* Ehrenberg, 1832 strain "oblonga", side view, from Mytopo Lake. (17) *Difflugia oblonga* Ehrenberg, 1832 strain "tenuis", side view, from Mytopo Lake. (18) *Difflugia oblonga* Ehrenberg, 1832 strain "spinosa", side view, from Mytopo Lake. (19) *Difflugia oblonga* Ehrenberg, 1832 strain "lanceolata", side view, from Bond Lake. (20) *Difflugia oblonga* Ehrenberg, 1832 strain "triangularis", side view, from Island Lake. (21, 22) *Cucurbitella tricuspis* (Carter 1856), side views, from Mount Hope Lake and Island Lake. (23) *Difflugia protaeiformis* Lamark 1816 strain "protaeiformis", side view, from Mytopo Lake. (24) *Difflugia protaeiformis* Lamark 1816 strain "protaeiformis", side view showing heavy agglutination on neck typical of many specimens, from Mytopo Lake. (25) *Difflugia protaeiformis* Lamark 1816 strain "acuminata", side view, from Mew Lake. (26) *Difflugia protaeiformis* Lamark 1816 strain "acuminata", side view, from Swan Lake. (27) *Difflugia protaeiformis* Lamark 1816 strain "claviformis", side view, from Mytopo Lake. (28) *Difflugia glans* Penard, 1902 strain "glans", side view, from Jake Lake. (29) *Difflugia glans* Penard, 1902 strain "magna", side view, from Jake Lake. (30) *Difflugia glans* Penard, 1902 strain "distenda", side view, from Jake Lake.

Table 2
Radiocarbon ages obtained from Haynes Lake. OxCal v4.1.7 Bronk Ramsey (2011); r:5; Atmospheric data from Reimer et al (2009).

Sample ID	Laboratory number	Description	Depth (cm)	^{14}C age	Calibrated age ranges (yr BP)
					IntCal09
HYNL_69–70 cm	Beta-275540	Bulk organic	69–70	3330 ± 40	3553 ± 89
DC969	UBA-17176	Plant macrofossil	74–75	2017 ± 35	1972 ± 88
C1-2-3-5	Beta-260148	Bulk organic	97	4900 ± 40	5652 ± 66
C1-2-36-38	Beta-260149	Bulk organic	130	5350 ± 40	6108 ± 108
DC971	UBA-17177	Plant macrofossil	130–131	4226 ± 37	4695 ± 68
C1-3-53-54		Bulk organic	241–242	11110 ± 60	12955 ± 197

impacts adjacent water bodies (Evanylo and Beegle, 2006). As the reconstructed phosphorus values in the post settlement period remain relatively low from the AD 1870 s (~32 cm) through to ca. AD 1968 (14 cm), this may suggest that that farmed areas near Haynes Lake were primarily used as pasture supporting a relatively modest livestock herd, or to grow hay which requires only periodic tilling.

Beginning ~AD 1972 (12 cm) there was a dramatic increase in inferred OP that persisted through to ~AD 1985 (7 cm) that must have led to significant eutrophication of Haynes Lake. This major increase in P could have been the result of either wastewater discharge or agricultural runoff. As argued below, the most likely cause of the P increase was agricultural runoff from fields, treated by chemical fertilizers.

By 1959 all North American detergents were comprised of up to 50% P, a significant proportion of which made its way into lakes and rivers as sewage outflow, seepage, or runoff (Vollenweider 1968). By 1983 over 1.8 billion kg of P were used annually in the US for detergents alone

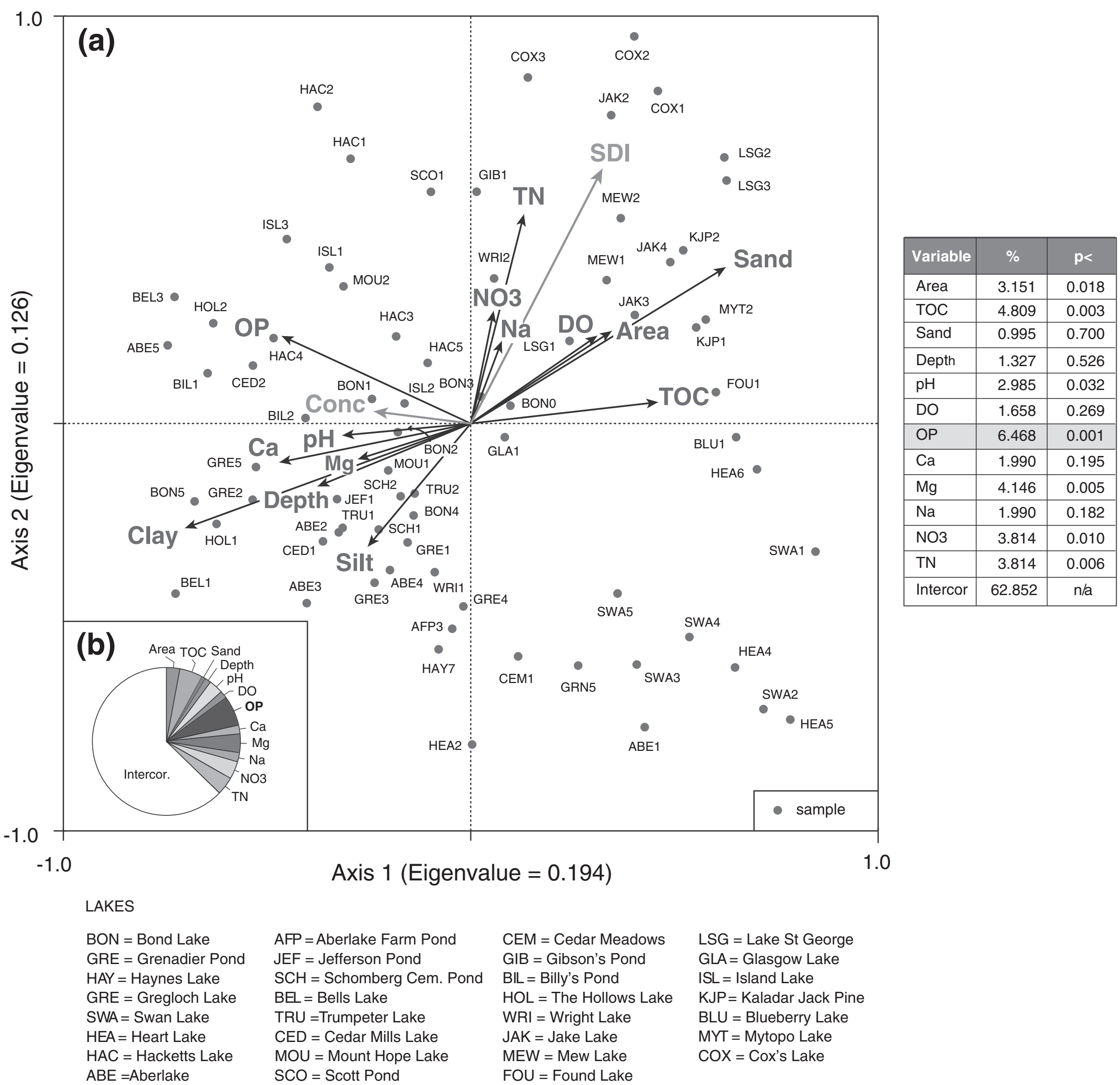


Fig. 5. Redundancy analysis (RDA) sample-environment bi-plot for the 73 samples that yielded statistically significant arcellacean populations. The environmental variables have been chosen by forward selection ($p<0.05$). SDI (Shannon Diversity Index) and Conc. (arcellacean concentration) are plotted passively. TOC = Total organic carbon, DO = dissolved oxygen; TN = total nitrogen; NO3-nitrate; Temp = temperature. b) Partial canonical correspondence analyses (pRDA) results showing the percentage variance in the arcellacean dataset explained by the measured environmental variables and intercorrelation P values for each variable are shown in the table.

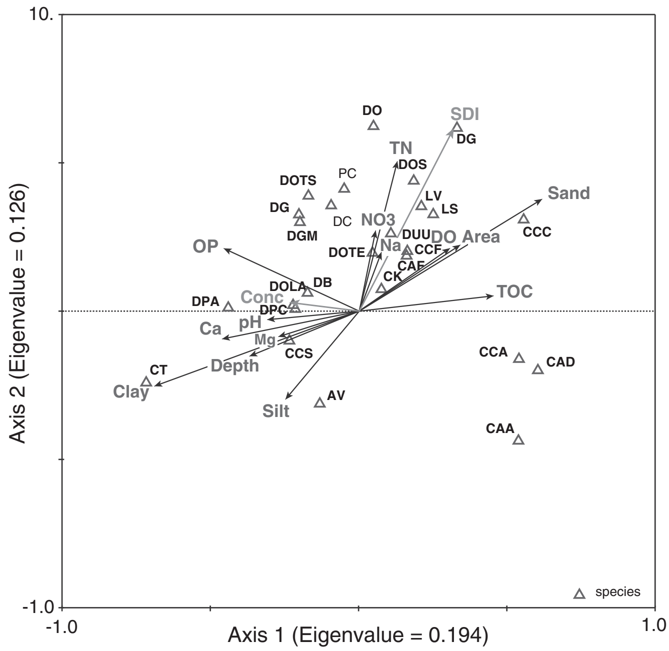


Fig. 6. RDA species–environment bi-plot. The environmental variables have been chosen by forward selection ($p<0.05$). AV = *Arcella vulgaris*; CAA = *Centropyxis aculeata* "aculeata"; CAD = *Centropyxis aculeata* "discoides"; CCA = *Centropyxis constricta* "aerophila"; CCC = *Centropyxis constricta* "constricta"; CCS = *Centropyxis constricta* "spinosa"; CK = *Cyclopyxis kahli*; CT = *Cucurbitella tricuspis*; DB = *Difflugia bidens*; DC = *Difflugia corona*; DOB = *Difflugia oblonga "bryophila"*; DOLA = *Difflugia oblonga "lanceolata"*; DOO = *Difflugia oblonga "oblonga"*; DOLS = *Difflugia oblonga* "linearis"; DOS = *Difflugia oblonga* "spinosa"; DOTE = *Difflugia oblonga* "tenuis"; DOTS = *Difflugia oblonga* "triangularis"; DPA = *Difflugia protaeiformis* "acuminata"; DPC = *Difflugia protaeiformis* "claviformis"; DUU = *Difflugia urceolata* "urceolata"; LS = *Lesquereusia spiralis*; LV = *Lagenodifflugia vas*; PC = *Pontigulasia compressa*. Environmental variable symbols are defined in the Fig. 5 caption.

(Wetzel, 2001). As one kg of P can grow 1500 kg of algae the damage caused by excess P inputs to lacustrine environments was tremendous (Beeton, 1971). Although the US continued to use P in detergents the Canada Water Act of 1970 resulted in a drastic reduction in laundry detergent P (Knud-Hansen, 1994) so households near the lake were unlikely to have contributed to a P increase in Haynes Lake that began ~AD 1972. A more likely source was chemical inorganic fertilizers, which contain P as a significant macronutrient (Smil, 2000; Binford, 2006). The use of these inorganic fertilizers became wide spread in the decades following WWII (Mills and Jones, 1996; Erisman et al., 2008). The P component in inorganic fertilizers binds strongly with many elements, compounds and the surfaces of clay minerals in soil but is rapidly released for biological update as these P-bearing minerals dissolve (Mullins and Hansen, 2006). Rainwater flowing across a soil surface can thus easily mobilize P through dissolution and transport of soluble P, or erosion and direct transport of particulate P. In areas where P bearing inorganic fertilizer has been applied for many years leaching and subsurface lateral flow of P can also occur. As virtually all transported P is biologically available both soluble and sediment bound P can contribute to excessive growth of aquatic organisms leading to eutrophication (Mullins and Hansen, 2006).

There was a steady decline in inferred OP concentration in Haynes Lake after AD 1985 (7 cm) through to ~AD 1995 (3 cm), which most likely corresponds to reduced use of chemical fertilizers. There was a realization, beginning in the early 1980s that applying excessive amounts of chemical fertilizers have negative environmental impacts, provide no additional crop yields and can even be as detrimental as under fertilization; a waste of farmers time and money (Smil, 2000; Tilman et al., 2002). Beginning in ~AD 1995 (3 cm) there was a dramatic increase in P levels in Haynes Lake that by the time of the collection of the core in AD 2005 were inferred to be as high as those of the AD 1980s.

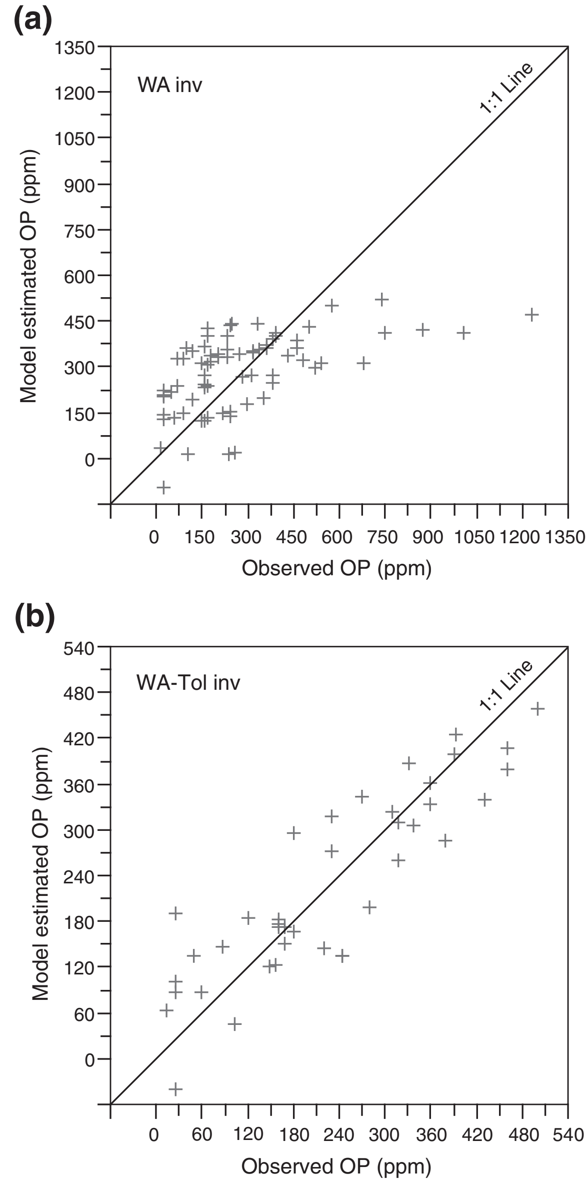


Fig. 7. Observed vs model estimated sedimentary (Olsen's) phosphorus; a) original dataset ($n=73$) run with WA inv model; b) screened dataset after removal of outliers (improved model — WA-TOL inv) ($n=40$). Model performance given in Table 1.

Although changing agricultural practices may have been a factor, another possible nearby source may have been the Diamond Back Golf and Country Club, which was built in AD 1993 up a slope and immediately to the north of Haynes Lake. In other areas, golf courses, which heavily fertilize their turf to maintain flawless fairways, have been identified as a major source of nutrient loading in adjacent aquatic ecosystems (Dillon and Winter, 2005). Development of this transfer function indicates that arcellaceans have utility as a proxy in the reconstruction of OP, and as such they promise to become a useful complement to other more established paleolimnological proxies like diatoms.

4. Conclusions

This research provides evidence that there is a quantitative link between arcellacean communities and OP, based on 73 sediment-water interface samples from 33 lakes. The lakes were selected to span a continuous P gradient and included urban to rural environments within the GTA, as well as lakes from undisturbed forested areas in eastern Ontario and Algonquin Park. Ordination (pRDA)

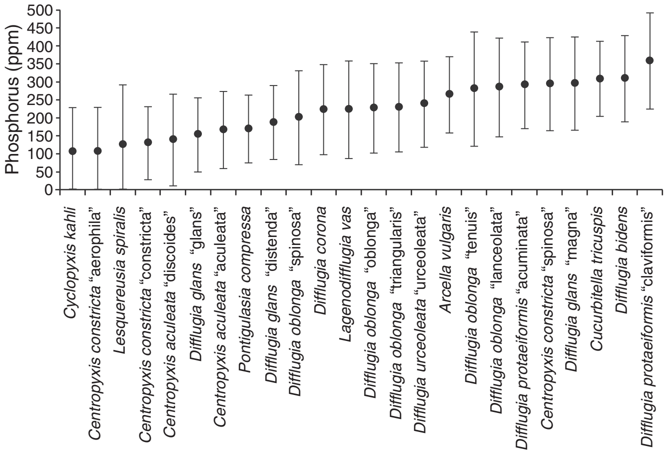


Fig. 8. Phosphorus (OP) tolerance and optima statistics for the 24 arcellacean species and strains present in statistically significant numbers in this study.

confirmed that OP has the greatest influence on assemblage variance, explaining 6.5% ($p<0.001$) of the total variance, providing further evidence of the sensitivity of arcellaceans to eutrophication (Roe et al., 2010). TOC (4.8%; $p<0.003$), TN (3.8%; $p<0.006$) and Mg (4.2%; $p<0.005$) also influenced the makeup of arcellacean community assemblages though.

To reduce the influence of TOC, TN, Mg and other variables the transfer function developed for OP was based on an improved training set comprising samples from 31 of the studied lakes. The best performing model was derived using WA-Tol with inverse deshrinking ($RMSEP_{jack}=77$ ppm; $r^2jack=0.68$). Testing of the WA-Tol inferred OP (ppm) reconstruction against contemporary arcellacean data from Teapot Lake, Innisfil Recreation Lake, Bell Lake and Cedar Mills Lake indicates that observed OP measurements fall within the bootstrap error range of the model-inferred OP value.

The model was subsequently applied to fossil arcellacean datasets from Haynes Lake on the ORM where the settlement history of the region is relatively well known, and using data from a core that is chronologically well-constrained. The results indicate that initial clearance of the area around Haynes Lake in the AD 1870s had little impact on OP concentrations in the lake. This indicates that initial land use of the area in the immediate lake catchment was either as pasture or was

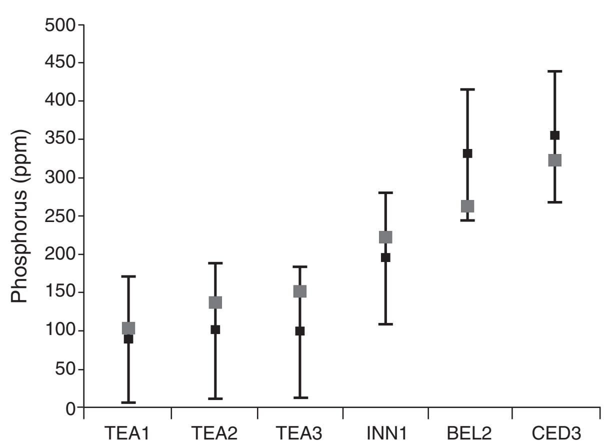


Fig. 9. Application of WA-Tol transfer function to three contemporary arcellacean samples from the Oak Ridges Moraine and surrounding area. Sample-specific prediction errors generated by 1000 bootstrap cycles show that the observed OP measurements lie within the bootstrap error of the model-inferred *P* value. Black square = WA Inv estimated P; gray square = measured *P* value. TEA = Teapot Lake; INN = Innisfil Recreation Lake; BEL = Bell Lake; CED = Cedar Mills Lake.

Table 3
^{210}Pb dates obtained from Haynes Lake Core C3-1. Atmospheric data from Reimer et al (2009); OxCal v4.1.7 Bronk Ramsey (2011); r:5.

Sample depth (cm)	^{210}Pb date (AD)
0.5	2002.9 ± 4.84
2.5	1996.6 ± 5.42
4.6	1990.0 ± 6.22
8.6	1980.2 ± 3.59
12.7	1972.4 ± 3.75
16.7	1963.1 ± 3.94
19.8	1954.7 ± 3.58
23.3	1941.5 ± 3.76
25.3	1927.9 ± 4.19
27.4	1917.0 ± 4.82
29.4	1893.0 ± 7.83
31.4	1877.9 ± 11.38
35.5	1829.1 ± 47.29

used to grow a perennial crop such as Timothy-grass for hay, as OP runoff from pastureland and the pre-settlement forests would have been minimal. After ~AD 1972 there was a dramatic increase in OP concentration in the lake that was probably related to the introduction of chemical fertilizers within the lake catchment. A rapid decline in inferred OP concentration after ~AD 1985 likely corresponds to a reduction in use of chemical fertilizers, possibly related to advances in crop science that permitted a much more precise determination of required application levels. Levels of inferred OP increased significantly again after ~AD1995, possibly related to the construction of a large golf course to the immediate north and upslope of Haynes Lake, where significant fertilizer use is required to maintain the grounds. The transfer function result indicates that arcellaceans are useful for reconstructing paleo-OP concentrations in lakes, providing an excellent complement other paleolimnological proxies (e.g. diatoms). Further research using more refined training sets targeted at other water property variables may result in their use to characterize other parameters as well (e.g. road salt contamination).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.palaeo.2012.05.028.

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